

## LIVE BIOLOAD DETECTION USING MICROPARTICLES

### CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/141,685, filed Dec. 31, 2008 and U.S. Provisional Patent Application No. 61/291,301, filed Dec. 30, 2009, which are incorporated herein by reference.

### BACKGROUND

[0002] Various tests are available that can be used to assess the presence of biological analytes in a sample (e.g. surface, water, air, etc). Such tests include those based on the detection of ATP using the firefly luciferase reaction, tests based on the detection of protein using colorimetry, tests based on the detection of microorganisms using microbiological culture techniques, and tests based on detection of microorganisms using immunochemical techniques. Surfaces can be sampled using either a swab device or by direct contact with a culture device such as an agar plate. The sample can be analyzed for the presence of live cells and, in particular, live microorganisms.

[0003] Results from these tests are often used to make decisions about the cleanliness of a surface. For example, the test may be used to decide whether food-processing equipment has been cleaned well enough to use for production. Although the above tests are useful in the detection of a contaminated surface, they can require numerous steps to perform the test, they may not be able to distinguish quickly and/or easily the presence of live cells from dead cells and, in some cases, they can require long periods of time (e.g., hours or days) before the results can be determined.

[0004] The tests may be used to indicate the presence of live microorganisms. For such tests, a cell extractant is often used to release a biological analyte (e.g., ATP) associated with living cells. The presence of extracellular material (e.g., non-cellular ATP released into the environment from dead or stressed animal cells, plant cells, and/or microorganisms) can create a high "background" level of ATP that can complicate the detection of live cells.

[0005] In spite of the availability of a number of methods and devices to detect live cells, there remains a need for a simple, reliable test for detecting live cells and, in particular, live microbial cells.

### SUMMARY OF THE INVENTION

[0006] In general, the present disclosure relates to articles and methods for detecting live cells in a sample. The articles and methods make possible the rapid detection (e.g., through fluorescence, chemiluminescence, or a color reaction) of the presence of cells such as bacteria on a surface. In some embodiments, the inventive articles are "sample-ready", i.e., the articles contain all of the necessary features to detect living cells in a sample. In some aspects, the inventive articles and methods provide a means to distinguish a biological analyte, such as ATP or an enzyme, that is associated with eukaryotic cells (e.g., plant or animal cells) from a similar or identical biological analyte associated with prokaryotic cells (e.g., bacterial cells). Furthermore, the inventive articles and methods provide a means to distinguish a biological analyte

that is free in the environment (i.e., an acellular biological analyte) from a similar or identical biological analyte associated with a living cell.

[0007] Methods of the present disclosure allow an operator to concentrate cells from a liquid sample and to detect an analyte associated with the cells. In certain embodiments, detection of the analyte may be an indicator of live cells including, in particular, live microbial cells in the sample. In some embodiments, the methods provide for the operator to measure the amount of a biological analyte in the sample. In some embodiments, the methods provide for the operator to, after a predetermined period of time during which an effective amount of a cell extractant is released from a composition into the liquid mixture, measure the amount of a biological analyte to determine differentially the amount of biological analyte from acellular material and from live cells in the sample. In some embodiments, the methods provide for the operator, within a first predetermined period of time, to perform a first measurement of the amount of a biological analyte and, within a second predetermined period of time during which an effective amount of cell extractant is released from the composition, perform a second measurement of the amount of biological analyte to detect the presence of live cells in the sample. In some embodiments, the methods can allow the operator to distinguish whether biological analyte in the sample was released from live plant or animal cells or whether it was released from live microbial cells (e.g., bacteria). The present invention is capable of use by operators under the relatively harsh field environment of institutional food preparation services, health care environments and the like.

[0008] In one aspect, the present disclosure provides a method of detecting cells in a sample. The method comprises providing a cell concentration agent, a hydrogel comprising a cell extractant and a liquid sample suspected of containing cells. The method further comprises contacting the liquid sample and the cell concentration agent for a period of time, isolating the cell concentration agent from at least a portion of the liquid sample, forming a liquid mixture comprising the isolated cell concentration agent and the hydrogel wherein the cell extractant is released into the mixture, and detecting a biological analyte. Optionally, the analyte can be detected at two or more discrete time points. In some embodiments, detecting a biological analyte comprises detecting a live cell. In some embodiments, detecting a biological analyte comprises using a detection system. In some embodiments, detecting a biological analyte comprises quantifying the analyte. In some embodiments, detecting a biological analyte comprises detecting ATP from a cell. In some embodiments, detecting a biological analyte comprises detecting the cell by genetic or immunological methods. In some embodiments, the method further comprises the steps of providing a somatic cell extractant and contacting the somatic cell extractant with cells from the sample.

[0009] In another aspect, the present disclosure provides a method of detecting cells in a sample. The method comprises providing a sample suspected of containing cells; a cell concentration agent; a hydrogel comprising a cell extractant; a detection article comprising a housing with two or more receptacles and an opening configured to receive the sample; means for isolating and transferring the cell concentration agent from a upper receptacle to a lower receptacle in the housing. The method further comprises contacting in a liquid medium the sample and the cell concentration agent in the upper receptacle of the housing. The method further com-